EXPRESS MAIL CERTIFICATE

EXPRESS MAIL No.: EL 645 877 797 US Deposited: **October 25, 2001**

I hereby certify that this correspondence is being deposited with the United States Postal Service Express mail under 37 CFR 1.10 on the date indicated above and is addressed to: Commissioner for Patents, Washington, D.C. 20231.

Ruth Montalvo

In the event that this paper is late filed and a necessary Petition for an Extension of Time is not concurrently filed herewith, please consider this as a Petition for the requisite extension of time, and to the extent not tendered by check attached hereto, authorization to charge the extension fee, or any other fee required in connection with this paper, to Deposit Account No. 50-1529.

JG-YY-5018/500569.20050

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Hiroshi SHINOKI, et al.	Group:	1656
Serial No.:	09/706,405	Examiner:	S. HOUTTEMAN
Filing Date:	November 3, 2000	Customer No.:	026418

For: FIXATION OF NUCLEOTIDE DERIVATIVES TO SOLID CARRIER

Commissioner for Patents
Washington, D.C. 20231

AMENDMENT

Sir:

In response to the Office Action mailed April 25, 2001, please amend the above-identified application as follows:

IN THE SPECIFICATION:

Please delete the paragraph at page 12, lines 30-36 and substitute therefor the following paragraph:

- - A nucleotide oligomer (3'-CTAGTCTGTGAAGTGTCTGATC-5', 22 mers, SEQ ID NO 1) having L-glutamylglycine at 3'-terminal and a fluorescent label (FluoroLink, Cy5-dCTP, available from Amasham Pharmacia Biotec Corp.) at 5'-terminal and D-glutamyl transferase (Oriental Yeast Co., Ltd., 11U) were dispersed in 1 L of an aqueous solution containing a triacetate buffer (300 mM, pH 6.0) at 1 x 10^{-M}. - -

Please delete the paragraph at page 13, line 27 through page 14, line 4 and insert therefor the following paragraph:

- - Target DNA fragments (GATCAGACACTTCACAGACTAG-5', 22 mers, SEQ ID NO 2) which had the fluorescent label Cy5-dCTP at 5'-terminal was dispersed in 20 L of an aqueous hybridization solution (mixture of 4xSSC and 10wt.% SDS solution) was spotted on the DNA chip (B'). The upper surface of the spotted solution was covered with a cover glass (for microscopic use) and then this was incubated in a moisture chamber at 60°C for 20 hours. Thus treated DNA chip was washed successively with a mixture of aqueous 0.1 wt.% SDS solution and aqueous 2xSSC solution, a mixture of aqueous 0.1 wt.% SDS solution and aqueous 0.2xSSC solution, and an aqueous 0.2xSSC solution. Thus washed DNA chip was centrifuged at 600 rpm for 20 seconds and then dried at room temperature. - -

Please insert at page 14, line 10 the sequence listing attached hereto.

IN THE CLAIMS:

Please cancel Claim 2 and amend Claims 1, 11 and 12 to read as follows.

1. (Amended) A method for fixing a plurality of nucleotide derivatives to a solid carrier which comprises bringing nucleotide derivatives having a reactive group at each

one terminal into contact with a solid carrier having thereon reactive groups in an aqueous phase in the presence of a transferase which is capable of producing a covalent bond by rearrangement of the reactive group of the nucleotide derivative and the reactive group of the solid carrier;

wherein the nucleotide derivatives are selected from the group consisting of oligonucleotides, polynucleotides and peptide-nucleic acids.

11. (Amended) A solid carrier to which oligonucleotides, polynucleotides or peptide-nucleic acids are attached, which is produced by the method of claim 1.

12. (Amended) A process of fixing complementary nucleic acid fragments contained in a sample liquid to a solid carrier which comprises the steps of:

bringing a sample liquid containing labelled nucleic acid fragments into contact with a solid carrier according to claim 11, whereby labelled nucleic acid fragments complementary to the oligonucleotides, polynucleotides or peptide-nucleic acids fixed to the solid carrier are combined with the oligonucleotides, polynucleotides or peptide-nucleic acids by hybridization; and

removing not-combined labelled nucleic acid fragments from the solid carrier.

REMARKS

As a result of the foregoing amendment, Claim 2 has been cancelled and Claims 1, 11 and 12 have been amended. Accordingly, Claims 1 and 3-12 are pending in this application.

In the Office Action, the Examiner has indicated that a substitute specification and claims is required since the specification originally filed in this application is poorly reproduced and difficult to read. Accordingly, Applicants have submitted herewith a substitute specification including the claims originally filed. Applicants have also submitted herewith formal drawings and respectfully request that the formal drawings be entered. No new matter has been added in the substitute specification, claims and formal drawings.

Also in the Office Action, the Examiner has issued a Notice to Comply with Requirements for Patent Applications Containing Nucleotide and/or Amino Acid Sequence Disclosures ("Notice to Comply") in which the Examiner asserts that the application fails to comply with the requirements set forth in 37 C.F.R.§§1.821-1.825. To comply with the requirements of 37 C.F.R.§§1.821-1.825, the Examiner indicates in the Notice to Comply that Applicants must provide a computer readable form (CRF) copy of the sequence listing, a paper copy of the sequence listing and a statement that the content of the paper and CRF copies are the same. Accordingly, Applicants have hereinabove amended the specification to include a sequence listing, a paper copy of which is attached, and have also submitted herewith a copy of the Notice to Comply as requested by the Examiner and a CRF copy of the sequence listing. Applicants respectfully submit that the paper and CRF copies of the sequence listing are identical and include no new matter.

As Applicants have added a sequence listing to the specification, Applicants have amended the specification at pages 12 and 13 to add sequence identifier numbers corresponding to the sequence listing. Applicants have also attached hereto a marked-up copy of pages 12 and 13 of the specification showing the changes in red ink. Applicants respectfully submit that no new matter has been added in the specification.

Also in the Office Action, the Examiner has rejected Claims 1-12 as being indefinite. In particular, the Examiner asserts that the recitation "nucleotide derivatives" in the claims is not clear. In response to this rejection, Applicants have hereinabove amended Claims 1, 11 and 12 to provide that the nucleotide derivatives are selected from a group including oligonucleotides, polynucleotides and peptide-nucleic acids. Support for this amendment is found in the specification at page 7, lines 12-14. Applicants have also attached hereto a copy of Claims 1, 11 and 12 as originally filed with the changes made herein shown in red ink. No new matter has been added in Claims 1, 11 and 12. Accordingly, Applicants respectfully submit that Claims 1 and 3-12 particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

In view of the foregoing, it is respectfully submitted that the claims are in condition for allowance and favorable reconsideration and prompt notice to that affect are earnestly solicited.

Respectfully submitted,

REED SMITH, LLP

October 25, 2001

By: _____

Samir R. Patel - Reg. No. 44,998
375 Park Avenue, 17th Floor
New York, NY 10152
Tel. (212) 521-5400

complete, the DNA chip is washed with an aqueous buffer solution containing a surface active agent, to remove a free(unfixed) sample DNA fragment. The surface active agent preferably is sodium dodecylsulfonate (SDS). The buffer solution may be a citrate buffer solution, a phosphate buffer solution, a borate buffer solution, Tris buffer solution, or Goods buffer solution. The citrate buffer solution is preferably employed.

The hybridization on the DNA chip is characteristic in that an extremely small amount of the sample or target DNA fragment is subjected to the analysis. In order to perform the desired hybridization appropriately, optimum conditions should be determined.

The present invention is further described by the following examples.

Example 1

[Example 1 -- Preparation of DNA chip]

The process for preparing a DNA chip utilized in this example is illustrated in Fig. 2. In Fig. 2, a slide glass is indicated by number 1, and GT means D-glutamyl transferase.

A slide glass (25 mm x 75 mm) was immersed in an ethanol solution of 2 wt.% aminopropyltriethoxysilane (available from Shin-etsu Chemical Industries, Co., Ltd.) for 10 minutes. Subsequently, the slide glass was taken out, washed with ethanol, and dried at 110°C for 10 min. Thus, a silane coupling agent-treated slide glass (A) was prepared.

A nucleotide oligomer (3'-CTAGTCTGTGAAGTGTCTGATC-5', SEQ ID NO 1 22 mers) having L-glutamylglycine at 3'-terminal and a fluorescent label (FluoroLink, Cy5-dCTP, available from Amasham Pharmacia Biotec Corp.) at 5'-terminal and D-glutamyl transferase (Oriental Yeast Co., Ltd., 11U) were dispersed in 1 μL of an aqueous solution containing a triacetate buffer (300 mM, pH 6.0) at 1×10^{-M}.

The resulting aqueous dispersion was spotted onto the silane coupling agent-treated slide glass (A) obtained above, and this was immediately kept at 25°C, 90%RH, for one hour. Thus treated slide glass was then washed successively twice with a mixture of aqueous 0.1 wt.% SDS (sodium dodecylsulfate) solution and aqueous 2xSSC solution (obtained by doubly diluting standard sodium chloride-citrate buffer solution (SSC)), once with the aqueous 2xSSC solution, and finally with distilled water. Thus washed glass slide was dried at room temperature, to give the desired DNA chip (B).

The fluorescence strength of the DNA chip (B) was measured using a fluorescence scanning apparatus. The fluorescence strength was 923. The fluorescence strength is well higher than a fluorescence strength 352 which was measured in a conventional DNA chip in which an oligonucleotide was fixed by electrostatic force. This means that the oligonucleotides are well fixed to the slide glass.

[Example 2 -- Detection of sample DNA fragments]

(1) Preparation of DNA chip

The procedures of Example 1 were repeated except for not attaching the fluorescent label to the 5'-terminal, to prepare a DNA chip (B').

(2) Detection of sample DNA fragments

Target DNA fragments (GATCAGACACTTCACAGACTAG-5', 22 mers) which had the fluorescent label Cy5-dCTP at 5'-terminal was dispersed in 20 μL of an aqueous hybridization solution (mixture of 4xSSC and 10 wt.% SDS solution) was spotted on the DNA chip (B'). The upper surface of the spotted solution was covered with a cover glass (for microscopic use) and then this was incubated in a moisture chamber at 60°C for 20 hours. Thus treated DNA chip was washed successively with a mixture of aqueous 0.1 wt.% SDS solution and aqueous 2xSSC solution, a mixture

SEQ ID NO 2

of aqueous 0.1 wt.% SDS solution and aqueous 0.2xSSC solution, and an aqueous 0.2xSSC solution. Thus washed DNA chip was centrifuged at 600 rpm for 20 seconds and then dried at room temperature.

The fluorescence strength of the DNA chip having been subjected to hybridization was measured using a fluorescence scanning apparatus. The fluorescence strength was 568. This means that the target DNA fragments are well fixed to the DNA chip (B').

What is claimed is:

1. A method for fixing a plurality of nucleotide derivatives to a solid carrier which comprises bringing nucleotide derivatives having a reactive group at each one terminal into contact with a solid carrier having thereon reactive groups in an aqueous phase in the presence of a transferase which is capable of producing a covalent bond by rearrangement of the reactive group of the nucleotide derivative and the reactive group of the solid carrier, wherein the nucleotide derivatives are selected from the group consisting of oligonucleotides, polynucleotides and peptide-nucleic acids.

2. The method of claim 1, wherein the nucleotide derivatives are those selected from the group consisting oligonucleotides, polynucleotides, and peptide-nucleic acids.

3. The method of claim 1, wherein the transferase is selected from the group consisting of carboxyl transferase, carbamoyl transferase, and aminoacyl transferase.

4. The method of claim 1, wherein the reactive group of the nucleotide derivative or the solid carrier is selected from the group consisting of amino, carboxyl, acyl, and carbamoyl.

5. The method of claim 1, wherein the reactive group of the nucleotide derivative or the solid carrier is selected from the group consisting of amino, aldehyde, epoxy, and carboxyl.

6. The method of claim 1, wherein the reactive group is attached to the nucleotide derivative via a linking group.

7. The method of claim 6, wherein the linking group is an alkylene group or an N-alkylamino-alkylene group.

8. The method of claim 1, wherein the reactive group of the nucleotide derivative is γ-amide group of a glutaminyl moiety attached to the nucleotide derivative, the reactive group of the solid carrier is an amino group attached to the carrier, and the transferase is transglutaminase.

9. The method of claim 8, wherein the amino group is attached to the solid carrier by bringing a silane coupling agent into contact with the carrier.

10. The method of claim 1, wherein the solid carrier is selected from a glass plate, a resin plate, a metal plate, a glass plate covered with polymer coat, a glass plate covered with metal coat, and a resin plate covered with metal coat.

11. A ~~nucleotide derivative fixed solid carrier~~ solid carrier to which oligonucleotides, polynucleotides or peptide-nucleic acids are attached, which is produced by the method of claim 1.

12. A process of fixing complementary nucleic acid fragments contained in a sample liquid to a solid carrier which comprises the steps of:

bringing a sample liquid containing labelled nucleic acid fragments into contact with a ~~nucleotide derivative attached~~ solid carrier according to claim 11, whereby labelled nucleic acid fragments complementary to the ~~nucleotide derivative~~ oligonucleotides, polynucleotides or peptide-nucleic acids fixed to the solid carrier are combined with the ~~nucleotide derivative~~ by hybridization; and

removing not-combined labelled nucleic acid fragments from the solid carrier.

oligonucleotides, polynucleotides or peptide-nucleic acids